KAROLINSKA INSTITUTET
– *a medical university* –

EXHIBIT 1

Department of: CLINICAL IMMUNOLOGY

Laboratory Journal No:

Name: [redacted]
HANS WIGZELL

Group:

Date: [redacted]
from to

Laboratory Journal

248909

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Study	Project no. / Study no.
Assembly of a synthetic gene coding for Fel d1 chain 1 using Taq	Project no.: Study no.: 29

PCR

Oligos 132, 133, 134, 135 10 µM

1 µl of each oligo 132-135
1 µl dNTP 10 mM
0.5 µl Taq
1 µl 10x Taq-buffer
3.5 µl H_2O
———
10 µl

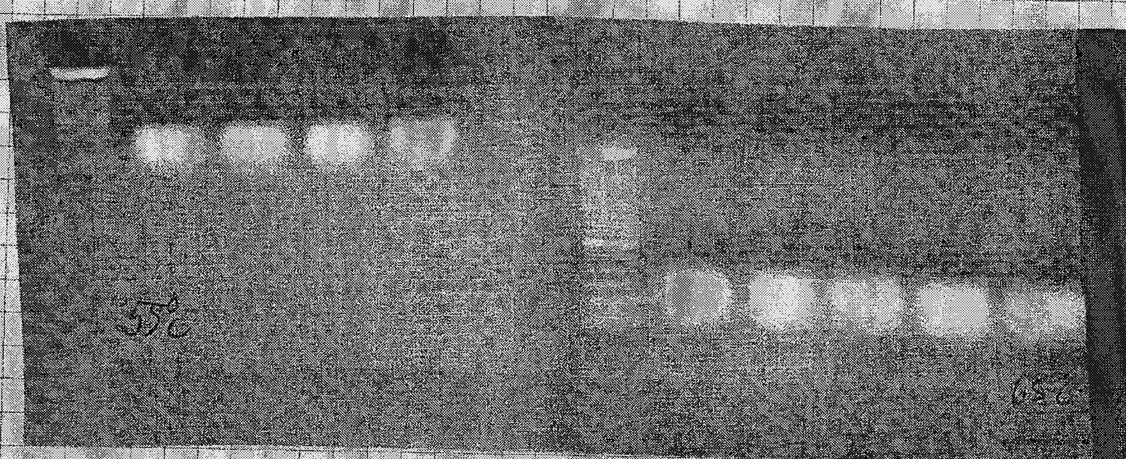
→ PCR Eppendorf program HANS1

94°C 1 min
annealing 55-65°C 1.5 min (grad)
elongation 68°C 2.0 min

30 cycles ending 10 min elongation

+4°C

Result: A strong band around 300 (exp. ~ 260)



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Laboratory Journal

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Study: Assembly of claim 2 Del d1

Project no.:

Study no.:

Primers 127-131 + 138

Using 3 different DNA polymerases Taq pfu+ Ampli Taq

Methods: "assembly claim 1"

expected band at 294

+300

to 248913 lane 1

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Study: Expression and purification of Fel d1 chain 1 and chain 2.

Project no.

Study no.

Fel d1 chain 1 (clone 42) and Fel d1 chain 2 clone 29 was ligated into pET 20b and electroporated into BL-21 DE3 pLysS after having been cut from pT7-Blue containing the correct sequence. (see binder HG1, Fel d1)
Sequencing of pET 42 / pET 29 was done accordig to standard protocol (ABI) and the result can be seen on the opposite side.

Both Fel d1 chain 1 (Fd1:1) and Fel d1 chain 2 (Fd1:2) was expressed according to standard protocol and purified on a thTrap (chelate) column loaded with $NiSO_4$.
Chain 1 was soluble, after ultrasonication chain 1 was found in 20 mM Tris HCl pH fraction, while chain 2 was found in the inclusion bodies after "washing" with 2M urea buffer + 20mM Tris HCl pH 8.0. The inclusion bodies were solubilized in 6M Guanidin, transferred to 6M Urea buffer (20mM Tris-HCl pH 8.0 + 0,5M NaCl) via 6 his HiTrap. Purification was done on FPLC

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~~Luft~~ sensibiliserade Blad4 ALLGOT II

Personnummer	kdnr	g.1	e1 prick	
340217-3235	29	0.47		Atopiker
370413-3218	59	11.4	3	Atopiker
380620-1236	58	9.2	3	
410317-3210	95	1.24		
420509-3215	102	2.21		
421013-3254	110	0.43		
430306-3277	118	1.3		
441118-3272	135	1.03	2	
471031-3273	172	1.07		
490710-3218	199	0.92		
500228-3217	203		2	
500515-3233	204	0.73		
510414-3231	215		2	
511020-3212	220	0.64	3	
531016-3232	243	0.37		
531106-7835	245	0.85	2	
540209-3214	253		2	
540823-9332	231	67.7	3	
550521-3213	277	28.5	2	Atopiker
560701-3216	283	0.68		
570415-3211	295	0.69		
[illegible]	317	0.82	2	
[illegible]	343		2	
[illegible]	352	2.09		
621119-3252	375		2	
630127-3212	380		2	
630703-3230	385	1.55		
640724-3234	398	0.47		
650523-3210	403		2	
650828-3212	406	0.76		
660703-3211	416	3.06	2	
671004-3256	422	25.5	3	Atopiker
680220-3213	426		2	
680520-3210	428	0,50		
690108-3235	434	21.45	3	Atopiker
730812-3251	452	5.33		
740306-0044	454	6.33	2	Atopiker
740313-3213	455	0.36		
750109-3210	456	17.1	3	

Σ 31 st.

C:\program\genesis\protocol\abs3\07NOV00W.004

```
LABSYSTEMS GENESIS V3.xx   Results [redacted]
Raw data filename          : c:\program\genesis\protocol\abs3\[redacted]
Processed by Protocol      : c:\program\genesis\protocol\abs3.prt
Plate layout file          : c:\program\genesis\protocol\abs3.plt
Reading type               : Dual Wavelength
Instrument version         : Multiskan RC V1.3.0
Filter 1                   : 405nm
Filter 2                   : 620nm
Lag time                   : 00:00:10
Interval between rdgs      : 00:00:10
Mix                        : YES
Mix RPM                    : High
Mix ON period              : 00:00:05
Mix OFF period             : 00:00:05
Comments:
Platta 2 45 min

Raw data values (calculated):
```

	1 3,5x	2 10x	3 3,5x	4 10x	5 3x	6 10x	7 3x	8 10x	9 3x	10 10x	11	12
A	0.042	0.372	0.327	1.058	2.079	1.363	2.475	0.128	0.149	0.053	0.192	0.197
B	0.048	0.340	0.394	1.826	1.801	1.119	2.448	0.156	0.152	0.098	0.120	0.193
C	0.049	[illegible]	0.347	0.877	1.325	1.066	[illegible]	0.148	0.138	[illegible]	[illegible]	[illegible]
D	0.008	0.008	0.008	0.007	0.008	0.007	0.008	0.008	0.008	[illegible]	0.008	0.008
E	0.006	0.008	0.008	0.008	0.008	0.009	0.007	0.008	0.008	0.007	0.008	0.008
F	0.006	0.008	0.008	0.009	0.009	0.008	0.008	0.009	0.007	0.009	0.008	0.008
G	0.007	0.007	0.007	0.007	0.008	0.009	0.008	0.007	0.009	0.005	0.007	0.008
H	0.008	0.007	0.008	0.006	0.008	0.008	0.007	0.008	0.010	0.010	0.008	0.008

(29) kontroll (59) (277) (422) (434) (29) (454)

ku/L 0,47 11,4 28,5 25,5 21,45 6,3

atopi ? 31

```
Comments:
Platta 3 45 min

Raw data values (calculated):
```

	1 3x	2 10x	3 3x	4 10x	5 3x	6 10x	7 3x	8 10x	9 3x	10 10x	11 3x	12 10x
A	0.049	0.057	0.188	0.112	1.395	0.817	0.537	0.253	0.297	0.088	0.178	0.095
B	0.046	0.053	0.316	0.095	1.403	0.689	0.643	0.245	0.135	0.063	0.146	0.117
C	0.050	0.051	0.178	0.056	1.137	0.449	2.304	0.958	0.114	0.020	0.158	0.103
D	0.051	0.086	0.182	0.105	1.182	0.776	2.463	0.219	0.179	0.085	0.145	0.172
E	0.054	0.132	0.068	0.059	0.851	0.411	2.408	1.092	0.114	0.028	0.148	0.084
F	0.324	0.042	0.056	0.234	0.897	0.533	2.477	1.118	0.111	-0.043	0.179	0.088
G	0.063	0.050	(0.387)	0.108	1.435	0.623	0.407	0.258	0.182	0.086	0.153	0.120
H	0.053	0.119	0.153	(0.250)	1.140	1.354	1.518	0.046	0.107	0.046	2.297	0.089

K1 (rows A–D), K2 (rows E–H)

29 59 277 422 434 457

Coating: A = 10 µg/ml
B = 5 µg/ml
C = 2,5 µg/ml
D = 1,25 µg/ml

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Study

Project no.

Study no.

Test of Fd 1 chain 1 clone 42 and
Fd 1 chain 2, clone 29 with 6 cat sensibilized
biopics from Algot Study

6 farmers from the ALGOT study, no 29, 59, 277, 422, 434 and 454, was diluted 3 times and 10 times resp. in PBS pH 7.4. A μ-titer plate was coated with:

Plate 1

A 10 μg/ml chain 1 ⟶ (All horizontal wells)
B 5 μg/ml —"—
C 2,5 μg/ml —"—
D 1,25 μg/ml —"—
E 10 μg/ml chain 2 ⟶
F 5 —"—
G 2,5 —"—
H 1,25 —"—

Plate 2

A 5+5 μg/ml chain 1+2 (a 1:1 mix of resp chain
B 2,5+2,5 —"— chain 1+2 with 5 μg/ml each)
C 1,25+1,25 —"— —"—

On the verikal rows the patients were added

1	2	3	4	5	6	7	8	9	10	11	12
↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓
Pacient 29		59		277		422		434		454	
3x	10x	3x	10x								

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ELISA conditions

Coating of Fd 1:1 and Fd 1:2 (100 µl/well) over the weekend in +4°C. ~~Serum~~ 4 times wash with "Tvättlösning"

Patients serum was added, 100 µl/well and incubated at +4°C o.n. Wash 4 times (Wallac ELISA-washer) with "Tvättlösning" and 100 µl Rabbit/human ~~anti~~ IgE diluted 1000 times in "Vår buffert"

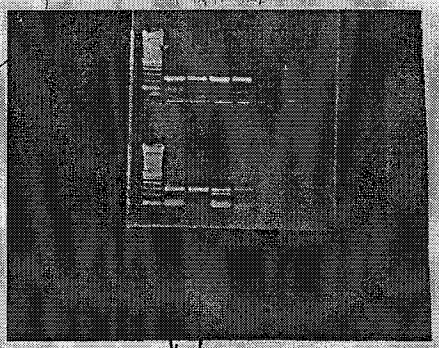
Inkubation 2 hours in RT on shaker, Wash 4 X "tvättlösning" and add 100 µl/well of Goat anti-rabbit-ALP konjugated (DAKO) for 1 h

Wash 4 times and add substrate 3 tablets/15ml of Vår buffert.

The result was read in ELISA reader after 45 min at 405 ~~nm~~ nm

Result: 2.5 µg/ml seem to be an adequate coating concentration for both chain 1 and chain 2. Mixing of the two chains can be done with coating concentrations 2.5 + 2.5 µg/ml

001114



(1a) 1µl template clone 11. 42 (1:1000)
2µl primer 176
2µl —"— 174
2µl 10× buff
1µl pfu
2µl dNTP (10mM)
10µl H_2O
20µl

(1b) Som ovan men, primer Taq polymeras

(2a) 1µl templ. (1:1000) klon 42
1µl templ —"— klon 29
2µl primers. 176, 174, 183
i övrigt som (1a)

(2b) som (2a) men Taq

(3a) 1µl templat klädja 2 (1:1000)
2µl 187
2µl 175
i övrigt som 1a

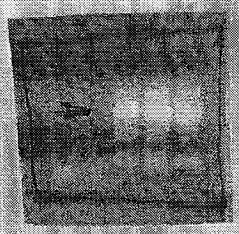
(3b) Som (3a) men Taq

(4a) 1µl templ. 1
1µl templ. 2
2µl primer 180
i övrigt som (3a)

(4b) Som (4a) men Taq

Putting clone 1 and 2 (1+2) together (001121)

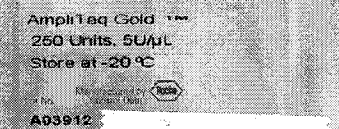
template 1a 6µl
template clone 29 1µl
1:10, 1:100, 1:1000
0,5µl primer 176
2µl —"— 183
2µl dNTP
3µl 10× buff.
1µl pfu
14,5µl H_2O
30µl



Result: One band of ~500bp which could be clone 1+2

The bands are cut out and purified on Qiagen

ligated with "perfectly blunt" cloning kit". 10 colonies are picked for miniprep and possibly sequencing



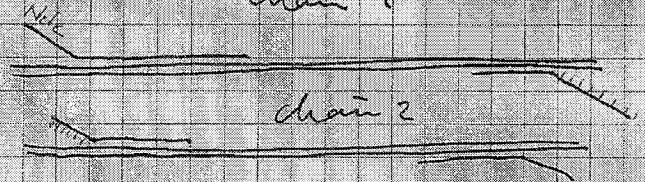
Study: Linking of (chain 1 and chain 2 (seamless)) with PCR Fel d1:1 and Fel d1:2

Project no.

Study no.

Aim. The aim of this ~~experiment~~ is to join the two chains of Fel d1 into one construct by PCR

Study outline The two sequenced chain of the major ~~chains~~ allergen of cat Fel d1 (chain 1, clone 42) and chain 2, clone 29) is joined with PCR i two steps as outlined below. In

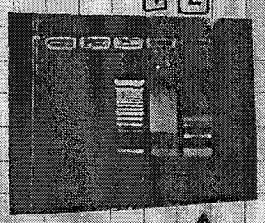


template 42 (plasmid Fel d1:1 in pET-Blue)

templ 29 (pl. Fel d1:2 in pET-B)

Result (see opposite side)

Good bands of expected size was seen for both chain 1 and chain 2. But (2a) + (2b) as well as (4a) and (4b) did not work. I will confirm by adding (1a) to ~~template~~ 29 and do PCR. (chain 1+2)



PCR:s [1] (1+2)

1µl templ. ch 1 (1:1000)

2µl 186

2µl 179

→ did not work

PCR primers 180, 181

[2] (2+1)

1µl template chain 1 (1:1000)

1µl chain 2 (1:1000)

2µl 181

2µl 175

2µl 180

1µl Pfu

3µl 10x buff

dNTP

H2O → ≤ 30µl

Cut band

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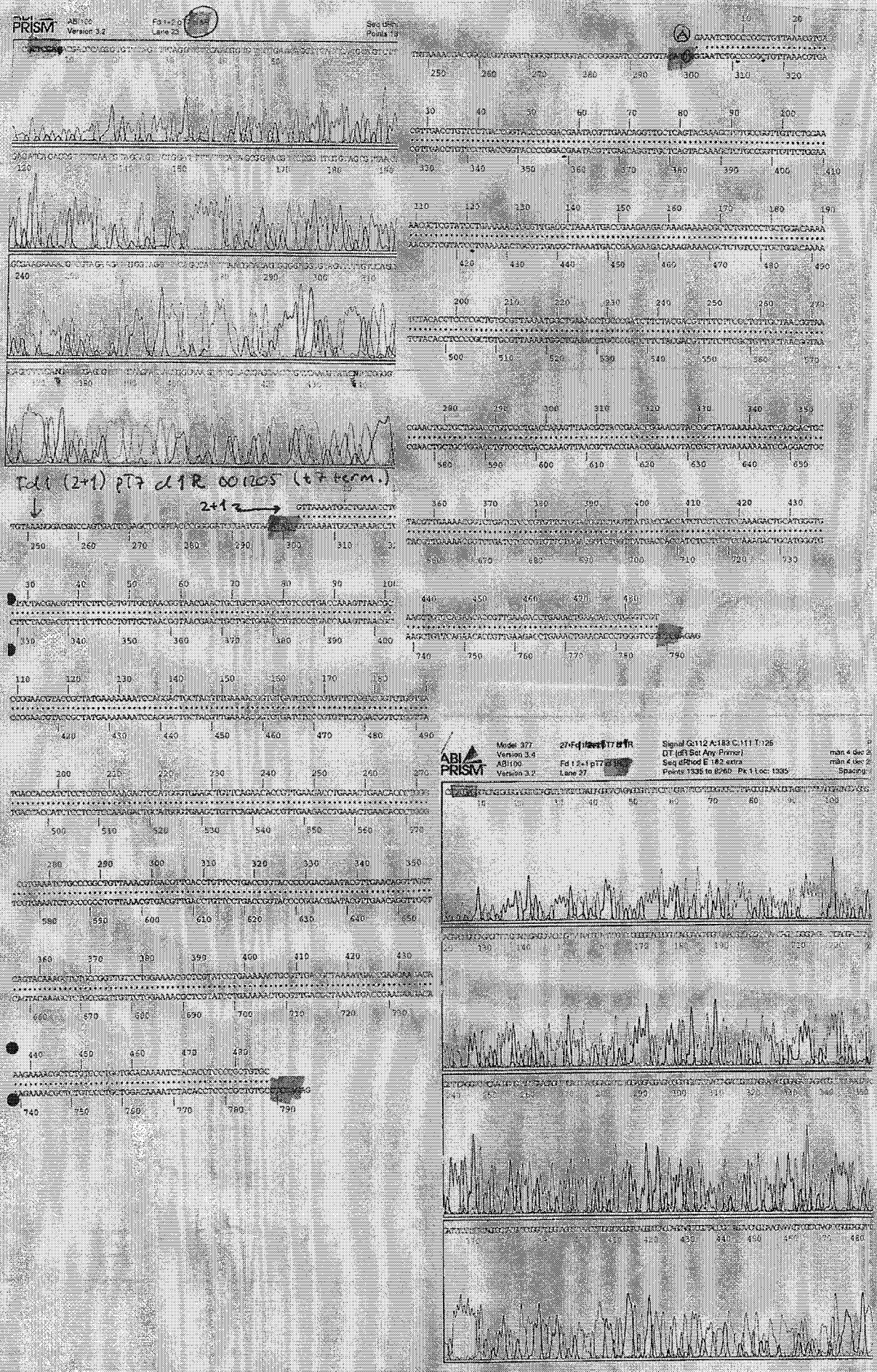
PRISM
ABI 100
Version 3.2
Fd1+2+1 pT7 cl1R 001205 (t7 term.)
2+1
ABI PRISM
Model 377
Version 3.4
ABI100
Version 3.2
Lane 27
Signal G:112 A:183 C:111 T:126
DT (dR Set Any Primer)
Seq dRhod E 182.extra
Points 1335 to 8260 Pk 1 Lcc: 1335
man 4 dec 2
min 4 dec 2
Spacing

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Study: Sequencing of 4 clones of each 1+2 and 2+1.

Project no.

Study no.

1+2	clone no		seq no	2+1		seq no	
Clone	1	=	(1)	Clone	1	(5)	OK seq red = prom.
	2	=	(2)		6	(6)	black = term.
	5	=	(3) OK seq		7	(7)	OK seq (prom ?)
	9	=	(4)		9	(8)	

4.8 µl vektor
1.2 µl primers
4 µl mix BD eller drhodamin

10 µl

+ 50 µl oil and

PCR på Perkin Elmer

96°C 30"
50°C 15"
60°C 4'
} 25 cykles

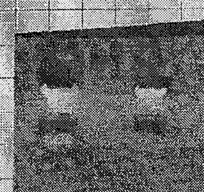
Unfortunately there was a scheduled power failure and the PCR-run was interrupted. Assume 7 more cycles which is done....

Samples are loaded on lanes 20 – 33 on ABI seq and named Fd1 1+2 pT7 clone 4F
– " – 4R
– " – 5F
– " – 5R
etc.

Cutting of clone 5 (1+2)
and clone 1 (2+1)
with Nde 1 and Xho 1
for ligation into pET 20b

20 µl plasmid miniprep (pT7 Blue)
2.4 µl 10x buff
0.3 µl BSA
0.7 µl Nde 1
0.7 µl Xho 1

Incubate 37°C shake for 2h.



→ Qiagel purif.

clone 1 (2+1) clone 5 (1+2)

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Study: Ligation of Ed 1, clone 1 (2+1) and clone 5 (1+2) into pET 20b+ and electroporation into BL21 plys

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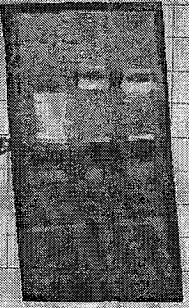
Study no.:

The fragments from Ed 1 (2+1) clone 1 and clone 5 (1+2) were ligen purified from 1% Agarose gel (248931)
A cleaved (Nde/Xho) pET 20b+ vector was used to ligate the fragments

Conditions

12 µl	~~vector~~ fragments
1.0 µl	vector
1.5 µl	10 mM ATP
1.8 µl	10x ligase buffer
1.7 µl	T4 ligase
18 µl	

Ligate +16°C o.n.
The ligate mix was electroporated into 50 µl BL21-plys electrocompetent cells. 1 µl ligate mix was added to thawed cells (on ice). Electroporation according to standard protocol. Growth on SOC medium for 60' 37°C shaker (300 rpm) and plated on Amp/CAM plates. One colony from each plate was picked and grown on LB Amp CAM liq. Mini prepped (Qiagen) and 25 µl of the (50µl) prep was cut with Nde and Xho. Result ~ 800bp



Both clones contain the insert !!

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Study: Purification of Fd 1 (1+2) klone 5 and Fd 1 (2+1) clone 1 over Ni⁺⁺ chelate Hi-Trap

Project no:

Study no:

1 Liter of Fd1 (1+2) and (2+1) (in pET 20b in BL-21 (plysS)) resp. LB-medium (CAM, Amp) was grown to OD 0.6 (600 nm) and induced with 0.4 mM IPTG. (see 248932)

Purification according to protocol. Both proteins were expressed as inclusion bodies and purified accordingly. Purification on FPLC as follows. After adsorption onto Ni-column wash with 6M Gua and wash also with 6 M Gua. The column is stuck to FPLC

Program:

0 conc % B 0 —— A = 6 M Urea

0 ml/min 5.0 ml/min B = 20 mM Imidazol

0 0.25 ml/min

0 port set 6.1 C = 500 mM Imidazol

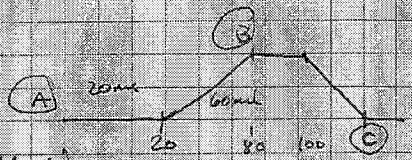
20 conc % B 0

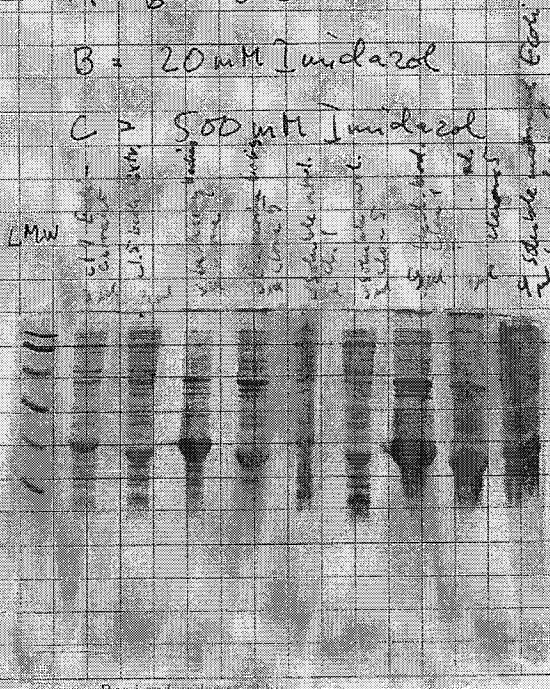
80 conc % B 100

100 conc % B 100

125 conc % B 0

125 port set 6.0





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